History of microbials and anti-microbials

Targets, spectrum, resistance, susceptibility, bacteriostatic vs bactericidal
Chemotherapeutic Agents

- Antibiotics
- Antifungals
- Antihelmintics
- Antivirals
- Antiprotozoal
- Anticancer drugs
The term *antibiotic* has its origin in the word antibiosis (i.e. against life). Antibiotics are chemical substances obtained from various species of microorganisms (bacteria, fungi, actinomycetes) that suppress the growth of other microorganisms and eventually may destroy them.

The probable points of difference amongst the antibiotics may be:

1) Physical  2) chemical  3) pharmacological properties  4) antibacterial spectra  5) mechanism of action.
Infection

- Infection is the colonization of the host organism with a microorganism like bacteria, parasite, virus, or even a macro organism like fungi and macro parasites such as worms and nematodes. The microorganism then will use the host resources to reproduce and grow that results in a disease.

- Host system normally use the immune system to fight against the invading organism, first by the innate immune system, then by the adaptive immune system.
Oldest antibiotics

1. Molded curd of soybean was used in Chinese folk medicine to treat boils and carbuncles.

2. Molded cheese was sued by Chinese and Ukrainian farmers to treat infected wounds
History of microbials discovery

- Bacteria are single-cell microorganism first identified in 1670s by van Leeuwenhoek following his invention of the microscope.

- The relation between bacteria and infection was not recognized until 1800++, the French scientist Pasteur and Joubert noticed that bacteria are crucial for milk fermentation and might be responsible for diseases. They also noticed that anthrax bacilli were killed if grown in culture with other specific bacteria.

- The Edinburgh surgeon Lister struggled to prove that surgeons are infecting their patients in operation theater. Thus he introduced the carbolic acid as operating ward antiseptic.

- Later in 1800+, Koch identified specific microorganisms that are responsible for specific diseases such as tuberculosis, cholera and typhoid.
History of anti-microbials discovery

- Most of the early compounds were antiprotozoals but little antibacterials were developed.

- In 1934, Proflavine was used as wound disinfectant in World War II, however, it was unsuitable for systematic use due to its high toxicity.

- In 1935, Prontosil was discovered as effective antibacterial agent in vivo. Later it found to be a prodrug that release sulfanilamide as the active metabolite.
In 1936, the first use of chemotherapy to combat infection was by Paul Ehrlich. Chemicals (magic bullets) were used at concentrations tolerated by the host to selectively inhibit microorganism proliferation (selective toxicity). Later in 1910, Paul developed synthetic toxic (antimicrobial) compound as arsenic complex called salvarsan which was effective against trypanosome.

Salvarsan is a mixture of dimer (A), trimer (B) and Pentamer (C) of 4-arsenio 2-amino phenol.
History of anti-microbials discovery

- The discovery of sulfonamides or sulfa drugs class of antibacterial agents was a breakthrough in the treatment of systematic bacterial infections.

- In 1940, penicillin (a bacterio-toxic fungal metabolite) was isolated from mold culture, even its effect was discovered earlier in 1929 by Alexander Fleming. The discovery revolutionized the fight against bacterial infection by proven more effective than sulfonamides.

- In 1944-1955, several antibiotics were isolated from microorganisms such as streptomycin & neomycin (aminoglycosides), chloramphenicol, chlortetracycline (tetracyclines), erythromycin (macrolides), valinomycin & bacitracin (cyclic peptides), cephalosporin C (β-lactams)
Streptomycin (aminoglycosides)

Vancomycin (peptidoglycans)

Bacitricin (cyclic polypeptide)

Chloramphenicol

Tetracyclin

Streptomycin (aminoglycosides)
History of anti-microbials discovery

- In 1952, synthetic antibacterial agents were developed such as isoniazid (anti-tuberculosis).

- In 1962, antibacterial quinolone of nalidixic acid (first generation) was developed followed by ciprofloxacin in 1987 (second generation).

**Figure 2.** Early history of antibiotics discovery and development.
History of anti-microbials discovery

Therefore, a substance is classified as an antibiotic if the following conditions are met:

1. It is a product of metabolism (although it may be duplicated or even have been anticipated by chemical synthesis).

2. It is a synthetic product produced as a structural analog of a naturally occurring antibiotic.

3. It antagonizes the growth or survival of one or more species of microorganisms.

4. It is effective in low concentrations.
A substance is classified as medicinal antibiotic if it has the previous conditions in addition to the following characters:

1. It must exhibit sufficient **selective toxicity** to be decisively effective against pathogenic microorganisms or neoplastic tissue, on the one hand, without causing significant toxic effects, on the other.

2. It must be **chemically stable** enough to be isolated, processed, and stored for a reasonable length of time without deterioration of potency and can be converted to other forms suitable for oral and parenteral uses.

3. The **rates of biotransformation** and elimination of the antibiotic should be slow enough to allow a convenient dosing schedule.
The bacterial cell

- The important feature for antibacterial agents is their selective action against bacterial (prokaryotic) cells than animal (eukaryotic) cells.

- Prokaryotic cells differ significantly from eukaryotic cells by having:
  
  > 1-10 µm length whereas eukaryotic length is 10-100 µm.
  > No nucleus.
  > Circular DNA, no chromosomal structure.
  > Most of the organelles are simpler than in eukaryotics.
  > Different biochemistry (e.g. synthesize vitamins)
  > Characteristic cell wall which differ from bacteria to another, but generally, it is thick and fatty envelope which protect the bacterial cell from lysis and invading by external environment.
Gram-positive and gram-negative bacteria

Gram-positive and Gram-negative bacteria differ in their cell wall composition. Gram-positive bacteria consist of 50-200 layers of peptidoglycan, while Gram-negative bacteria have only two layers.

Hans C. J. Gram, a Danish microbiologist, developed the Gram staining method (methyl violet-iodine) for staining bacteria so that they were more readily visible under the microscope.
The Gram stain is a staining procedure of great value in the identification of bacteria. The staining technique involves the addition of a purple dye followed by washing with acetone.

Bacteria with a thick cell wall (20–40 nm) absorb the dye and are defined as Gram-positive because they are stained purple.

Bacteria with a thin cell wall (2–7 nm) absorb only a small amount of dye, and the excess dye is washed out with acetone. These bacteria are then stained pink with a second dye (safranin) and are said to be Gram-negative.

- **Gram-positive bacteria**—these cells have a thick cell wall and are coloured purple.
- **Gram-negative bacteria**—these cells have a thin cell wall and are coloured pink.
Examples of important gram+ and gram- bacteria

<table>
<thead>
<tr>
<th>Organism</th>
<th>Gram</th>
<th>Infections</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Positive</td>
<td>Skin and tissue infections, septicaemia, endocarditis; accounts for about 25% of all hospital infections</td>
</tr>
<tr>
<td><em>Streptococcus</em> species</td>
<td>Positive</td>
<td>Several types—commonly cause sore throats, upper respiratory tract infections, and pneumonia</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Negative</td>
<td>Urinary tract and wound infections, common in the gastrointestinal tract, and often causes problems after surgery; accounts for about 25% of hospital infections</td>
</tr>
<tr>
<td><em>Proteus</em> species</td>
<td>Negative</td>
<td>Urinary tract infections</td>
</tr>
<tr>
<td><em>Salmonella</em> species</td>
<td>Negative</td>
<td>Food poisoning and typhoid</td>
</tr>
<tr>
<td><em>Shigella</em> species</td>
<td>Negative</td>
<td>Dysentery</td>
</tr>
<tr>
<td><em>Enterobacter</em> species</td>
<td>Negative</td>
<td>Urinary tract and respiratory tract infections, septicaemia</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Negative</td>
<td>An opportunist pathogen, can cause very severe infections in burn victims and other compromised patients, e.g. cancer patients; commonly causes chest infections in patients with cystic fibrosis</td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em></td>
<td>Negative</td>
<td>Chest and ear infections, occasionally meningitis in young children</td>
</tr>
<tr>
<td><em>Bacteroides fragilis</em></td>
<td>Negative</td>
<td>Septicaemia following gastrointestinal surgery</td>
</tr>
</tbody>
</table>
Bacterial nomenclature

**FIGURE A5.1** Bacterial nomenclature.

- **Cocci** (spherical)
- **Bacilli** (cylindrical)
- **Streptococci** (chains)
- **Staphylococci** (clusters)
Potential targets for antibacterial agents

Although the target and mechanism of action are unknown for many antibiotics, those known can be classified into the following:

- Cell metabolism (e.g. folate synthesis)
- Bacterial cell wall synthesis
- Cytoplasmic membrane
- Protein synthesis
- Nucleic acid synthesis

The targets and mechanism of action determine the antibiotic selectivity toward microorganisms than host cells.
Potential targets for antibacterial agents

- **Cell metabolism (e.g. folate synthesis)**
  - Disruption of bacterial cell metabolism is achieved by inhibiting enzyme-catalyzed reactions.
  - Such enzymes could be unique to bacteria or even present in host, however with differences in structure.
  - Sulfonamides are example of antimetabolites acting on dihydropteroate synthase that forms precursors for folic acid which is critical for nucleic acid synthesis. While trimethoprim act on folate reductase. Sulfonamides mimic Para-aminobenzoic acid (PABA) component of folic acid
  - \( \textit{dihydropteroate synthase} \rightarrow \text{Folic acid } \rightarrow \textit{folate reductase} \rightarrow \text{DHFA and THFA} \)
Potential targets for antibacterial agents

- **Bacterial cell wall synthesis**
  - Unlike eukaryotic cells, the bacterial cell has a cell wall which controls the size of bacterial cell against the osmotic pressure.
  - During cellular division new cell wall is created.
  - Any disruption of cell wall synthesis and repair leads to cell lysis.
  - Several agents derived from fungal (Penicillins, cephalosporins and glycoproteins o vancomycin) and bacterial source (bacitracin) act this way.
Potential targets for antibacterial agents

- **Cytoplasmic membrane**
  - The bacterial cell membrane is permeable to various compounds and has in/out gates
  
  - Affecting membrane permeability has fatal consequences.
  
  - Agents from bacterial source Polymyxins, Colistin act in this way.
  
  - Agent are more toxic if used systematically than those inhibiting cell wall
Protein synthesis

- Most of structural (e.g. peptidoglycans) and catalytic (e.g. enzymes) components of the cell are fully or partially made of proteins.
- Disruption of protein synthesis have disastrous effect on cell survival.
- Agents may inhibit protein synthesis at RNA transcription (Rifampicin), at ribosome (aminoglycosides, tetracyclines, macrolides, chloramphenicol).
- Structural difference between prokaryotic and eukaryotic ribosomes and polymerases improve selectivity, however the agents are toxic at high doses.
Potential targets for antibacterial agents

- **Nucleic acid synthesis**

Inhibition of nucleic acid transcription and replication prevents cell division and synthesis of essential proteins. Agents act this way include nalidixic acid and proflavine.
Bacterial resistance to antibacterial agents

- Resistance is the failure of microorganisms to be killed or inhibited by antimicrobial treatment.

- Resistance could be present before exposure to drug i.e. intrinsic (e.g. due to impermeability or lack of susceptible target) or developed after exposure i.e. acquired (e.g. due to genetic mutations or transfection by DNA plasmid).

- If two antimicrobial agents share the same target, resistance to the first affects the activity of other

- Resistance mechanisms include: ↓penetration, ↑efflux, ↑destruction, Δ target

- Persistence is bacterial survival within host cells, cysts or abscesses thus it is hard to be reached by antibiotics.
Plasmid may encode:

1. Enzymes that destroy the antibiotic
2. Enzymes that modify the antibiotics
3. Pumps that efflux antibiotics
4. Another copy of enzyme being targeted by antibiotic
Spectrum of antibacterial agents

- Antimicrobials that inhibit wide range of bacterial genera belonging to both gram-positive and gram-negative cultures are termed ‘broad spectrum’ such as tetracyclines.

- Antimicrobials that inhibit only few bacterial genera are termed ‘narrow spectrum’ such as the glycopeptides of vancomycin which act on gram-positive & anaerobics.

- The earlier terms; ‘broad spectrum’ and ‘narrow spectrum’ are less meaningful nowadays due to emergence of microbes resistant to single and multiple agents.
Identification of pathogen and antibiotic selection

- **Empirical-based therapy**: specific bacteria are associated with specific diseases
  - UTI $\leftarrow$ gm-ve *E. Coli*
  - Skin infection $\leftarrow$ gm+ve *Staph aureus*

- **Experimental-based therapy**: culturing microorganism on growth media, identifying genus and species followed by *in vitro* assays to determine MIC & MBC

Susceptibility test

Inhibition zone $= f(\text{type, conc})$
Antibiotic-sensitivity testing. Petri dishes were spread-inoculated with *Staphylococcus albus* (white growth) or *Micrococcus luteus* (yellow growth) before antibiotic assay "rings" were placed on the agar surface. The coloured disks at the end of each spoke of the rungs are impregnated with different antibiotics. Clockwise from the top (arrow) these are: Novobiocin, Penicillin G, Streptomycin (white disk), Tetracycline, Chloramphenicol, Erythromycin, Fusidic acid (green disk) and Methicillin. Clear zones of suppression of bacterial growth around the individual antibiotic disks are evidence of sensitivity to these antibiotics.
Bactericidal vs. bacteriostatic

- Depending on the concentration being used, the antibiotic can act on bacteria as bactericidal (i.e. kill) or bacteriostatic (i.e. halt growth and replication).

- While for most antibiotics the bactericidal concentrations are achievable in vitro, only few antibiotics can be used at bactericidal concentrations in vivo due to toxicity issues.

- Gentamycin: bacteriostatic $\times 2$ or $\times 4$ bactericidal (safe to be used as bactericidal)

- Tetracycline: bacteriostatic $\times 40$ bactericidal (toxic if used as bactericidal)

- Bacteriostatic antibiotics retard the logarithmic growth of bacteria in order to give time for immune system to deal with it.
Combination therapy

✓ Combinations of antibiotics would broaden the antimicrobial spectrum.

✓ Combination of bactericidal antibiotics such as β-lactams and aminoglycosides are used for urgent treatments before pathogen identification.

✓ Combination of bacteriostatic antibiotics such as macrolides and sulfonamides are commonly used for Upper RTI by Haemophilus influenzae

✗ Combination of bacteriostatic (e.g. tetracycline) and bactericidal (β-lactams) is antagonistic.
Effect of serum protein binding on antibiotic activity

- The protein-bound antibiotic is not readily available for treatment of infection.

\[
\text{Available amount} = \text{total amount} - \text{protein bound amount}
\]

- Antibiotics tightly bound to plasma proteins can not be used to treat deep tissue infections

- Antibiotics not significantly bound to plasma proteins or easily released from the proteins have relatively shorter half-life.
Quinolones
Rifampicin
Penicillins
Cephalosporins
Sulfonamides (on metabolic enzymes)
Aminoglycosides
Tetracycline
Chloramphenicol
### Chemical classification of antibiotics

- Chemical classification of antibiotics is usually of limited value due to high chemical variability of antibiotics.
- Structurally similar antibiotics derived from different microorganisms may have similar mechanism of action.

<table>
<thead>
<tr>
<th>Cl.</th>
<th>Classification</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>Sulfonamides</td>
<td></td>
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<tr>
<td>II.</td>
<td>β-Lactams: include penicillins and cephalosporins</td>
<td></td>
</tr>
<tr>
<td>III.</td>
<td>Aminoglycosides: amino sugars such as streptomycins, kanamycins, neomycins, gentamycins</td>
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<tr>
<td>IV.</td>
<td>Polypeptides: such as tyrothricin and polymyxin</td>
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<tr>
<td>V.</td>
<td>Macrolides: large lactone ring</td>
<td></td>
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<tr>
<td>VI.</td>
<td>Tetracyclines:</td>
<td></td>
</tr>
<tr>
<td>VII.</td>
<td>Fused ring systems</td>
<td></td>
</tr>
<tr>
<td>VIII.</td>
<td>Lincomycins</td>
<td></td>
</tr>
<tr>
<td>IX.</td>
<td>Polyenes: Antifungal such as nystatin and amphotericins</td>
<td></td>
</tr>
<tr>
<td>X.</td>
<td>Unclassified antibiotics</td>
<td></td>
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</tbody>
</table>
The sulfonamides are bacteriostatic when administered to humans in achievable doses.

They inhibit the enzyme dihydropteroate synthase, an important enzyme needed for the biosynthesis of folic acid derivatives and, ultimately, the thymidine required for DNA.

They do this by competing at the active site with p-aminobenzoic acid (PABA), a normal structural component of folic acid derivatives.

Humans have no dihydropteroate synthase, which explains sulfonamides selectivity for bacterial cells.

Indeed, the antimicrobial efficacy of sulfonamides can be reversed by adding significant quantities of PABA into the diet.